Remarks

Reconsideration of this Application is respectfully requested.

Claims 183, 189 and 195 are sought to be amended and claims 43-45, 94-126 and 135-182 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. Support for the amendment to claims 183, 189 and 193 can be found, for example, in the specification at page 9, line 25 to page 10, line 22, page 32, lines 15-29, and page 37, line 26 to page 41, line 27. Upon entry of the foregoing amendment, claims 183-200 are pending in the application, with claims 183, 189 and 195 being the independent claims.

It is believed that this amendment will put the case in condition for allowance or better form for consideration on appeal. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 112

The Examiner rejected claims 183-200 under 35 U.S.C. § 112, first paragraph, because the specification allegedly does not enable any person skilled in the art to make and use the invention commensurate in scope with these claims. (*See* Paper No. 15, pages 2-3.) It was the Examiner's position that

> the specification, while being enabling for an isolated polypeptide comprising an amino acid sequence identical to Ser (69) - Ser (208) of SEQ ID NO:2, does not reasonably provide enablement for polypeptides having 90-97% amino acid sequence identity to said sequence for the reasons of record in paper #12 as applied to previously examined claims 127-134.

(Paper No. 15, pages 2-3.) Applicants respectfully traverse this rejection as it may apply to the pending claims.

In particular, the Examiner indicated that Applicants' assertion that mere unpredictability of the result of the experiment is not a consideration for determining whether experimentation is undue was not persuasive. (*See* Paper No. 15, page 3.) The Examiner further contended that

> Applicant is arguing that as long as one can test for an activity, it is not routine experimentation to make any polypeptide and screen for activity. This argument completely ignores many of the factors of *In re Wands*, including the lack of examples in the specification, the lack of guidance in the specification and the prior art (as no polypeptides which differ from SEQ ID NO:2 have been generated), the number of embodiments encompassed by the claims, and the unpredictability of the art to which the instant claims are directed.

(Paper No. 15, page 3.)

Regarding the concern expressed in the Office Action about the number of embodiments encompassed by the claims, Applicants note that independent claims 183, 189 and 195 are directed to polypeptides at least 90%, at least 95% and at least 97% identical to Ser (69) - Ser (208) of SEQ ID NO:2, respectively, wherein the polypeptides retain the activity of binding to an antibody that specifically binds a polypeptide consisting of the amino acids Ser (69) - Ser (208) of SEQ ID NO:2. As such, not just "any" polypeptide is

encompassed by the present claims. Not only must almost all of the amino acid residues be substantially identical to the N-terminal deletion mutant KGF-2Δ33, they must also possess a specific activity.

In addition, Applicants' previous argument did not "completely ignore" many of the *In re Wands* factors. Rather, at page 6 of the previous reply, Applicants were asserting that mere unpredictability of the result of an experiment is not a consideration in determining whether an amount of experimentation is undue.

In this regard, the situation in the present application is analogous to that in *Ex parte Mark*, 12 USPQ2d 1905 (BPAI 1989). In particular, the reasoning set forth in the present Office Action is similar to the Examiner's reasoning in *Ex parte Mark*. In *Ex parte Mark*, the relevant claims were directed to a method for producing DNA encoding a synthetic mutein of any protein by substituting Cys by other amino acids, wherein the mutein had the biological activity of the parent protein. The claims were rejected as being nonenabled, based on the prior art disclosure that 8 Cys muteins of two different proteins lacked or were substantially reduced in the biological activity of the parent proteins. The Examiner reasoned that:

> it would require undue further experimentation to construct . . . the *innumerable muteins* encompassed by the instant claims . . . and to screen the muteins produced for any of those which exhibit biological activity after modification.

Id. at 1906 (emphasis added). The Examiner further asserted that there was an established unpredictability as to modifying Cys residues to produce biologically active muteins.

Upon appeal, the Board of Patent Appeals and Interferences reversed the enablement rejection. The Board's holding was *not* based on any "predictability" that a particular Cys mutein would retain function, but on the fact that determining which Cys muteins retained

function required only routine experimentation. The evidence showed that some Cys residues were essential and could not be mutated (8 Cys residues of IFN-β and CSF-1), while other Cys residues were not essential (some Cys residues of IFN-β, IL-2, and TNF) and could be mutated successfully without reducing or destroying activity. *Id.* at 1906. The Board specifically found that: "[o]ne skilled in the art is clearly enabled to *perform such work* as needed to determine *whether* the cysteine residues of a given protein are needed for retention of biological activity." *Id.* at 1907 (emphasis added).

Thus, although it could <u>not</u> *be predicted* beforehand that the Cys residues in a previously uncharacterized protein were nonessential, the evidence indicated that at least some proteins do contain nonessential Cys residues, and additional ones could be identified using routine assays. Therefore, contrary to the statements in the Office Action, predictability is not required for enablement of the full scope of the present claims. Further, Applicants provide evidence below similar to the evidence deemed persuasive by the board in *Ex parte Mark*, to show that the claimed method is fully enabled.

The Examiner stated that "[i]n order to use the claimed invention, the polypeptides which are claimed would need to retain the activity (immunogenic or biological) of the polypeptide which has the amino acid sequence of SEQ ID NO:2 in order for one of ordinary skill in the art to use what is claimed. . . . The specification provides no guidance as to which amino acids (i.e. structural elements) of the native proteins are critical to the biological/immunological activity or which amino acids could be altered without destroying these activities." (Paper No. 15, pages 3-4.)

As discussed below, on the basis of the specification and knowledge in the art regarding protein mutants in general and fibroblast growth factor mutants in particular,

Applicants respectfully assert that it would not require undue experimentation to generate KGF-2Δ33 muteins that have antibody generating activity.

The specification provides guidance to one of ordinary skill as to which amino acids are more or less likely to be important for activity in the claimed method. The specification describes KGF-2Δ33 as being a member of the fibroblast growth factor (FGF) family and as containing amino acid which are conserved within the family members. (*See* Specification, page 5, lines 1-4 and Figure 2.)

In particular, the specification discloses

> substitutions of serine for cysteine at amino acid positions 37 and 106 and 150. An uneven number of cysteins means that at least one cysteine residue is available for intermolecular crosslinks or bonds that can cause the protein to adopt an undesirable tertiary structure. Novel KGF-2 proteins that have one or more cysteine replaced by serine or e.g. alanine are generally purified at a higher yield of soluble, correctly folded protein. Although not proven, it is believed that the cysteine residue at position 106 is important for function. This cysteine residue is highly conserved among all other FGF family members.

(Specification, page 52, line 24 to page 53, line 3.)

On the basis of studies involving FGF's, conserved Cys residues are known to be important for function. *See, e.g.,* Rinas *et al.*, "Cysteine to serine substitutions in basic fibroblast growth factor: effect on inclusion body formation and proteolytic susceptibility during in vitro refolding," *Biotechnology 10*:435-440 (1992). Applicants also note that Figure 1 shows the positions of the Cys residues in KGF-2.

Also contrary to the Examiner's assertion, the specification does provide, at page 32, lines 19-27, at page 40, lines 7-16, at page 53, line 20 to page 54, line 4 and Figure 4 the antigenic/hydrophilic regions of KGF-2. Five of these antigenic regions fall completely

within amino acids 69 to 208 of SEQ ID NO:2. One of ordinary skill in the art, using the guidance provided in the specification, would know not to alter at least one of the antigenic regions disclosed in the specification in order to obtain a polypeptide useful for generating antibodies.

Further, even though only one use needs to be enabled, Applicants have provided, at Figure 2, an alignment of KGF-2 with other fibroblast growth factors. One of ordinary skill in the art would know not to alter the residues which are conserved across these molecules in order to obtain a KGF-2 variant with the claimed biological activity.

In addition, the specification discloses, for example, on page 45, several polypeptides falling within the scope of the claims. The specification also provides guidance, on page 51, for particular amino acid substitutions which may be made in the amino acids 69 to 208 of SEQ ID NO:2. Numerous examples of KGF-2Δ33 polypeptides with amino acid substitutions are provided in Example 22, at page 152 to page 160. Further, the specification demonstrates that a polypeptide consisting of amino acids 63 to 208 of SEQ ID NO:2 (also known as KGF-2Δ28), which falls within the scope of the claims, is biologically active. (*See*, *e.g.*, specification at page 121, lines 6-8; and page 127, lines 20-24.) Thus, the specification provides ample direction for one of ordinary skill to make and use the polypeptides of the invention.

Additionally, the specification discloses preferred amino acid substitutions of KGF-2Δ33 which are predicted not to affect structure or function. In some cases, these substitutions are predicted to improve or change function to a desired effect. For example, the substitutions of cysteine for serine or alanine are generally purified at a higher yield. (*See* Specification, page 52, lines 24-29.) Furthermore, conservative substitutions which are

predicted to preserve protein folding and structure are listed on pages 51-52 of the specification. In addition, charged amino acids are preferred to be substituted with charged or neutral amino acids. The resulting molecules result in less aggregation and prevent reduced activity. (*See* Specification, page 50, lines 4-21.) Therefore, the specification does provide ample guidance for generating muteins that would likely function in the claimed method.

Moreover, Applicants submit that a number of studies have analyzed the tolerance of proteins to amino acid substitutions. For example, Gayle *et al.* generated over 3,500 IL-1α muteins by random mutagenesis. *See* Gayle *et al.*, *J. Biol. Chem. 268*:22105-22111 (1993). Multiple mutations were examined at every possible amino acid. The results showed that:

> Most of the molecule could be mutated with little effect on either [binding or biological] activity. Combining the data from saturation mutagenesis and site-directed mutagenesis, alterations at only 39 positions resulted in proteins with modified activity ratios. Most of the other 139 residues, or 75% of the molecule, may not contribute significantly to the biological activity of the molecule . . . allowing a wide variety of amino acids to be substituted with little effect on activity. . . . Only 24 unique DNA sequences, out of more than 3,500 examined, produced a protein that displayed a significant difference in activity from wild-type. . . . This represents less than 0.7% of the mutants examined.

Id., p. 22109.

Similar results involving IL-1α had previously been shown by Gronenborn *et al.*, who analyzed receptor binding activity of six IL-1α muteins each containing a single amino acid alteration. *See* Gronenborn *et al.*, *FEBS Lett. 231*:135-138 (1988). They observed that

"six different mutants were tested for receptor binding activity and showed no alteration with respect to wild-type protein." *Id.*, p. 135.

Zurawski *et al.* randomly generated 1090 muteins within residues 41-142 of IL-2, such that there was an average of 11 different amino acid substitutions per naturally occurring amino acid residue. *See* Zurawski *et al.*, *EMBO J. 12*:5113-5119 (1993). The muteins were assayed for specific activity. The investigators found that, in the 149 amino acid mIL-2 protein, only "23 residues are important for interaction with IL-2R, 18 residues are presumed to be part of the structural core, and three additional residues are important for structure" and "together with data from terminal deletion analysis, 98 mIL-1 residues (or 65% of the protein), were assigned as relatively unimportant residues." *Id.*, pp. 5114, 5115, and 5117.

Thus, as discussed above, Gayle *et al.* demonstrated that less than 1% of the 3,500 IL-1α muteins generated had a significant alteration of activity, and Zurawski *et al.* demonstrated that 98 IL-2 residues (or over 65% of the protein) were unimportant to function. Therefore, contrary to the suggestion in the Office Action, Applicants assert that, in general, proteins are resilient to modification and retain functional activity notwithstanding numerous amino acid substitutions, deletions and/or insertions.

Moreover, at the effective filing date, random mutagenesis was one of several methods known in the art for generating FGF muteins. There were known rules for making amino acid substitutions/deletions/insertions, and sequence similarity to previously described FGF's, including KGF, could have been used for predicting peptide regions likely to be important for function. *See*, *e.g.*, Bare, *et al.*, "Effect of cysteine substitutions on the mitogenic activity and stability of recombinant human keratinocyte growth factor," *Biochem.*

Biophys. Res. Commun. 205:872-879 (1994); Zhu *et al.*, "Glu-96 of Basic Fibroblast Growth Factor Is Essential for High Affinity Receptor Binding, Identification by Structure-Based Site-Directed Mutagenesis," *J. Biol. Chem. 270*:21869-21874 (1995); Wong *et al.*, "Analysis of Putative Heparin-binding Domains of Fibroblast Growth Factor-1, Using Site-Directed Mutagenesis and Peptide Analogues," *J. Biol. Chem 270*:25805-25811 (1995); Arakawa *et al.*, "The importance of Arg40 and 45 in the mitogenic activity and structural stability of basic fibroblast growth factor: effects of acidic amino acid substitutions," *J. Protein Chem. 14*:263-274 (1995); Burgess *et al.*, "Structure-function studies of FGF-1: dissociation and partial reconstitution of certain of its biological activities," *Mol. Reprod. Dev. 39*:56-60 (1994).

As of the filing date of the instant application, there was a high level of skill in the field of protein chemistry and molecular biology. Techniques were available for routinely making polypeptides and generating antibodies that bind these polypeptides. The invention could be practiced with readily available starting materials using methods that were well known in the art on the priority date of the instant application. Like the monoclonal antibody art discussed in *In re Wands*, practitioners making the polypeptides of the invention are prepared to screen for antibody binding activity. The Examiner has provided no objective evidence showing that making the claimed polypeptides and assaying for antibody binding activity were not routine to one of skill in the art at the time the invention was made.

In addition, the Examiner asserted that

> Applicant argues that the specification teaches antigenic regions of polypeptide of SEQ ID NO:2 and that "one of ordinary skill in the art, using the guidance provided in the specification, would know not to alter at least one of the antigenic regions disclosed in the specification in order to

> obtain a polypeptide useful for raising antibodies". This argument is not persuasive because antibodies are generally, raised to polypeptides which are injected into animals. The instant specification fails to teach how the antigenic regions would be affected by amino acid substitutions/deletions/insertions in that such modifications would alter the folding of the protein, and therefore, create and/or destroy antigenic sites. These modifications would necessarily generate new proteins which would more likely than not have new and/or different antigenic sites, thereby resulting in antibodies which may or may not recognize the native protein.

(Paper No. 15, page 4.)

The present claims are directed to polypeptides comprising an amino acid sequence at least 90%, 95% or 97% identical to Ser (69) - Ser (208) of SEQ ID NO:2, wherein the polypeptides bind to an antibody that specifically binds a polypeptide consisting of the amino acids Ser (69) - Ser (208) of SEQ ID NO:2. Accordingly, the claims require that the polypeptides of the invention retain immunological activity.

The specification discloses at page 32, lines 19-27, at page 40, lines 7-16, at page 53, line 20 to page 54, line 4 and in Figure 4 the antigenic/hydrophilic regions of KGF-2. "[A]ntigenic polypeptides or peptides that can be used to generate KGF-2-specific antibodies include the following [six antigenic areas]." (Specification, page 40, lines 7-16.) In addition, five of these antigenic regions fall completely within amino acids 69 to 208 of SEQ ID NO:2. Moreover, it is well known that "[amino acid c]hanges are preferably of minor nature, such as conservative amino acid substitutions that do not significantly affect folding or activity of the protein." (Specification, page 51, lines 16-17.) The specification further provides examples of conservative amino acid substitutions known to those skilled in the art. (*See* Specification, page 51, line 20 to page 52, line 7.) Consequently, one of ordinary

skill in the art, using the guidance provided in the specification, would know which regions to avoid modifying in order to obtain a polypeptide useful for generating antibodies as well as suitable amino acid substitutions should modifications be made.

The Examiner further asserted that

> Applicant asserts at page 10 of the response that pages 152-160 of the specification provide numerous examples of amino acid substitutions. However, a review of this section of the specification reveals that although point mutations are indicated, the mutants have never been tested to determine if biological activity is retained. These "examples" are not sufficient to provide guidance for making modifications to the polypeptide of SEQ ID NO:2 because it is still not know [sic] if biological activity is maintained or not.

(Paper No. 15, page 5.)

Applicants point out that an applicant need not have actually reduced the invention to practice prior to filing. *See Gould v. Quigg*, 822 F.2d 1074, 1078, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987) ("The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it.")(quoting *In re Chilowsky*, 229 F.2d 457, 461, 108 USPQ 321, 325 (CCPA 1956)). That is, the specification need not contain a working example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *See In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). Nevertheless, the specification *does* provide examples of specific residues for point mutations, thereby providing the necessary guidance to those skilled in the art. Determining whether a polypeptide containing one or more of the disclosed mutations can generate antibodies would be merely routine to those skilled in the art.

Because only an enabling disclosure is required, Applicants need not describe all actual embodiments.

The Examiner further contended that Applicants' argument that those skilled in the art are prepared to screen for antibody activity was unpersuasive because,

> while some screening is not undue, the necessity to screen each and every mutant encompassed by the claims without a reasonable expectation that any one embodiment will function in a manner to make it useful is undue. . . . The claims recite some degree of sequence identity to SEQ ID NO:2, but as no guidance in the specification is provided for which amino acids should be altered and which amino acids should be left unchanged, such modifications are purely random and the claimed proteins are merely a "wish to know".

(Paper No. 15, page 5.)

Applicants respectfully submit that a "reasonable expectation" of success in generating a polypeptide that will "function in a manner to make it useful" is not the standard for determining whether experimentation is undue. "[If to fulfill the requirements of 112, first paragraph, an applicant's] disclosure must provide guidance which will enable one skilled in the art to determine, with reasonable certainty before performing the reaction whether the claimed product will be obtained, . . . then all 'experimentation' is 'undue' since the term 'experimentation' implies that the success of the particular activity is uncertain." *In re Angstadt*, 537 F.2d 498, 503 (CCPA 1976). The statutory enablement requirement is satisfied if the specification "adequately guides the worker *to determine*, without undue experimentation, which species among all those encompassed by the claimed genus possess the disclosed utility." *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991) (emphasis added).

As previously demonstrated, the specification provides an abundance of guidance

"for which amino acids should be altered and which amino acids should be left unchanged." In addition, even if the modifications were purely random, Applicants have provided evidence that numerous randomly-generated muteins retain biological activity.

Applicants further submit that the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *See Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984)("Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is not a function of the claims to specifically exclude . . . possible inoperative substances . . . .'")(quoting *In re Dinh-Nguyen*, 181 USPQ 46, 48 (CCPA 1974)); *In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971) ("We agree that appellants' claims are not too broad . . . just because they read on even a large number of inoperative embodiments, since it seems to be conceded that a person skilled in the relevant art could determine which conceived but not-yet-fabricated embodiments would be inoperative with expenditure of no more effort than is normally required . . . .").

In summary, even assuming, *arguendo*, that the Examiner has established a *prima facie* showing that the full scope of the claims is not enabled, Applicants respectfully submit that they have rebutted this showing by providing sufficient evidence that one skilled in the art would be able to make and use the claimed invention using the specification and/or the art as a guide.

In view of the facts set out above, it would not have required undue experimentation to practice the claimed invention as of the priority date. As such, Applicants assert that the presently claimed invention is fully enabled. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with Markings to Show Changes Made

In the Claims:

Claims 183, 189 and 195 were amended as follows:

183. (Once amended) An isolated polypeptide comprising an amino acid sequence at least 90% identical to Ser (69) - Ser (208) of SEQ ID NO:2, wherein said polypeptide binds to an antibody that specifically binds a polypeptide consisting of the amino acids Ser (69) - Ser (208) of SEQ ID NO:2.

189. (Once amended) An isolated polypeptide comprising an amino acid sequence at least 95% identical to Ser (69) - Ser (208) of SEQ ID NO:2, wherein said polypeptide binds to an antibody that specifically binds a polypeptide consisting of the amino acids Ser (69) - Ser (208) of SEQ ID NO:2.

195. (Once amended) An isolated polypeptide comprising an amino acid sequence at least 97% identical to Ser (69) - Ser (208) of SEQ ID NO:2, wherein said polypeptide binds to an antibody that specifically binds a polypeptide consisting of the amino acids Ser (69) - Ser (208) of SEQ ID NO:2.

Claims 43-45, 94-126 and 135-182 were canceled without prejudice to or disclaimer of the subject matter contained therein.